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14. ABSTRACT

Deployed Navy dolphins are exposed to infectious agents and pollutants in environments that may compromise their health and system mission. Challenges dolphins experience during deployments include: transport to different time zones, changes in water temperature, contaminated water, varying acoustic levels, and exposure to wild marine mammal populations. These challenges (confinement/restraint, thermal stress, pollutants, and auditory stress) have been shown to cause immunosuppression in other mammals. Our laboratory has developed and is continuing to develop dolphin-specific markers and assays to assess immunocompetence in cetaceans. These markers and assays were used to evaluate sound as a stressor and looked at the effects of sound level and duration on dolphins as part of the "Temporary Threshold Shift" studies conducted by scientists at the Navy. Moreover, developed reagents and assays were used to assess immunocompetence in wild bottlenose dolphins. The neural-immune tests developed and adapted for bottlenose dolphins do show changes in both captive and wild animals depending on stress (e.g. duration of sound exposure), geographic location and disease state. These tests will be useful for monitoring health in both captive and wild dolphins as well as useful in assessing efficacy of vaccines, and the monitoring of the impact of the environment and various stressors on dolphin health.

15. SUBJECT TERMS
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FINAL REPORT

GRANT #:N00014-04-1-0816

PRINCIPAL INVESTIGATOR: Tracy Romano

INSTITUTION: Mystic Aquarium & Institute for Exploration (MAIFE)

GRANT TITLE: Continued Investigation of Immune Competence in Navy Marine Mammals: Implications for Health, Viability and Mission Readiness

AWARD PERIOD: 15 September 2004 - 31 January 2006

OBJECTIVE(S): To continue standardization of available dolphin-specific immunological assays and reagents; to assess the effects of environmental deployment challenges on the immune system and autonomic activation; to continue development of dolphin-specific immunological reagents and assays; to compare Navy dolphin neural-immune parameters with wild dolphins.

APPROACH: The approach for the above objectives utilized peripheral blood from both captive and wild dolphins. Blood samples were obtained opportunistically during routine procedures and exams from Navy dolphins (on average once per month) and sent to MAIFE. Blood was obtained from wild bottlenose dolphins as part of the Health and Risk Assessment (HERA) of bottlenose dolphin populations project (Harbor Branch Oceanographic Institute and the National Ocean Service). Plasma, serum, total white blood cells or separated mononuclear cells were isolated and used in reagent development, assay validation and/or health assessments. A select set of the same blood samples were also sent to a collaborator's laboratory to run in parallel to establish validation and inter-laboratory comparisons using the same as well as different techniques.

ACCOMPLISHMENTS:

Functional Cellular Assays

1. Natural Killer Cell Assay

We have adapted a flow-cytometry based functional assay to measure Natural Killer cell activity in dolphin peripheral blood. This assay was applied to Navy dolphins opportunistically for optimization. Additional blood samples will be needed to complete the optimization process.

2. Neutrophil and Monocyte Function (Phagocytosis and Respiratory Burst)

We have adapted and developed an assay utilizing flow cytometry to examine dolphin neutrophil and monocyte function including assessment of phagocytosis and respiratory burst. This newly developed assay required only 3 mls of blood instead of 30 mls which is important when obtaining blood samples from Navy animals and wild bottlenose dolphins. Moreover, both phagocytosis and respiratory burst could be measured simultaneously from the same blood sample. This assay has been optimized in our laboratory and was used to assess neutrophil and monocyte function in both Navy and wild bottlenose dolphins.

3. Immunophenotyping of Dolphin Lymphocyte Subsets
Through the antibodies that our laboratory and colleagues have developed and characterized we were able to quantify dolphin lymphocyte subsets in the peripheral blood including T, B and T helper lymphocytes and class II-positive cells utilizing flow cytometry. This assay was used on wild dolphin samples as well as sound/stress studies in Navy dolphins.

4. Neurohormones
High performance liquid chromatography with electrochemical detection has been used to measure and quantify dolphin catecholamines (Norephinephrine, Epinephrine, Dopamine) in collaboration with ARUP Laboratories (Salt Lake City, Utah). Radioimmunoassays have been adapted to measure and quantify dolphin stress hormones such as cortisol and aldosterone (in collaboration with Dr. Lee Berk at UC Irvine and Steve Lamb at Cornell University). Neurohormones were measured as part of the dolphin sound/stress studies.

Measurements of Dolphin Immunocompetence Before and After Stress
In addition to the development of dolphin-specific assays and reagents, opportunistic samples were obtained in conjunction with the Navy's Temporary Threshold Shift studies which examined the effects of sound on dolphin hearing. We were able to obtain blood samples before and after a dolphin was exposed to sound of different durations (follow-on to a previous effort) to determine if sound duration had an effect on neural-immune measures.
Wild Dolphin Population Studies
Due to our studies on Navy dolphins, we were given the opportunity to participate in related studies investigating dolphin stress and immune function in wild bottlenose dolphins. The most recent study focused on capture/release studies of dolphins in Charleston Harbor, SC and the Indian River Lagoon, FL.

CONCLUSIONS: The assays that we have developed and or adapted are all feasible assays to use to assess health given the constraints of obtaining blood samples from cetaceans (both captive and wild). The results from the sound/stress study showed immediate differences between the control and sound groups with regard to T cell proliferation and iron, with stronger effects observed in the 4 second duration group than in the 8 second duration group. Longer-term differences (after 24 hours of sound exposure or control) were detected in globulin levels, which decreased 24 hours after a control but increased after a sound experiment. The immune data from the wild bottlenose dolphins suggests that alterations in immunity may be associated with disease state, that immunity does vary between the locations and by gender and is associated with various contaminants found in the animals. Whether these alterations in immunity result in greater susceptibility and subsequent disease manifestation or are a result of the disease itself are not clear at the present time.

SIGNIFICANCE: The neural-immune tests developed and adapted for bottlenose dolphins do show changes in both captive and wild animals depending on stress (e.g. duration of sound exposure), geographic location and disease state. These tests will be useful for monitoring health in both captive and wild dolphins as well as useful in assessing efficacy of vaccines, and the monitoring of the impact of the environment and various stressors on dolphin health.

PATENT INFORMATION: No patents have been filed.

AWARD INFORMATION: Two students (Ashley Linton, undergraduate and Mandy Keogh, Masters Student) were awarded travel awards to present research funded by this award at the 2005 meeting of the International Association for Aquatic Animal Medicine. Ashley Linton was also awarded the best overall student presentation in the undergraduate category.

PUBLICATIONS AND ABSTRACTS: (for total period of grant):

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